Synthesis of N-(2-chloro-5-methylthiophenyl)-N'-(3-methyl-thiophenyl)-N'-[$^3H_3$]methylguanidine, {[$^3H_3$]CNS-5161}

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Short Title: Synthesis of [$^3H_3$]CNS-5161

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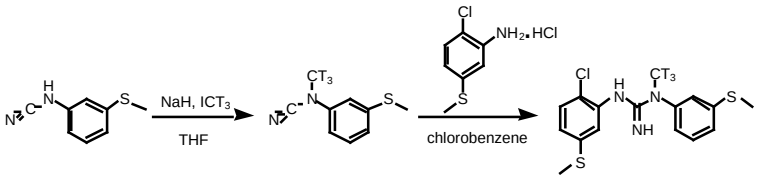
Graphical Abstract:



Synthesis of N-(2-chloro-5-methylthiophenyl)-N'-(3-methyl-thiophenyl)-N'-[$^3H_3$]methylguanidine, {[$^3H_3$]CNS-5161}

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Summary

The preparation of the title compound, [$^3H_3$]CNS-5161, was accomplished in three steps starting with the production of [$^3H_3$]iodomethane (CT_3I). The intermediate N-[$^3H_3$]methyl-3-(thiomethylphenyl)cyanamide was prepared in 77% yield by the addition of CT_3I to 3-(thiomethylphenyl)cyanamide, previously treated with sodium hydride. Reaction of this tritiated intermediate with 2-chloro-5-thiomethylaniline hydrochloride formed the guanidine compound [$^3H_3$]CNS-5161. Purification by HPLC gave the desired labeled product in an overall yield of 9% with greater than 96% radiochemical purity and a final specific activity of 66 Ci $mmol^{-1}$.

Keywords: CNS-5161, tritium, NMDA

Introduction

Glutamate, the major excitatory neurotransmitter in the brain, is thought to be involved in normal memory formation and brain plasticity as well as in pathological states such as stroke, head injury, epilepsy and several neurodegenerative and psychiatric disorders.[1-7] Glutamate interacts with several receptors, but a key role has been ascribed to the NMDA receptor, which is a ligand-gated ion (calcium) channel. Binding of glutamate to its binding site on the extracellular side of the transmembrane structure results in channel opening and influx of calcium into the cell. Glycine is a required cofactor. Noncompetitive antagonists (e.g., PCP, MK-801, CERESTAT; Figure 1) for the receptor have been identified and are thought to be "use dependent" ligands, (i.e. they bind to a site inside the channel when the receptor is activated by glutamate).[8] Therefore, increases in binding of agents of this class could be direct indicators of excessive glutamate release in the brain, a process implicated in excitotoxic neuronal death following brain ischemia. Excitotoxicity has also been suggested to contribute to neuronal death in neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.[1, 3, 4, 7] Due to its perceived importance in numerous crucial physiological and pathological states, this receptor/channel has been the target

of intensive research efforts aimed, among other things, at developing an appropriate radioligand for imaging of this receptor in vivo. Most of these efforts have not met with success.[9-12] In search of novel structures with promising attributes for in vivo imaging, we chose CNS-5161, a compound with high affinity and selectivity to the NMDA receptor channel[13], as a candidate for tritium labeling and further characterization.

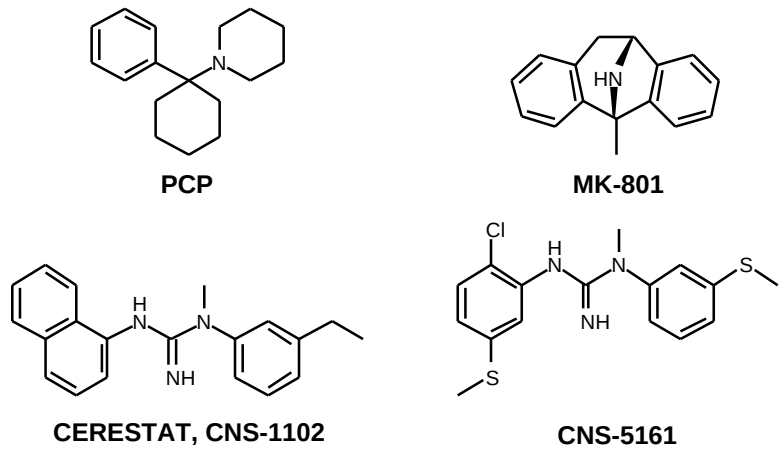
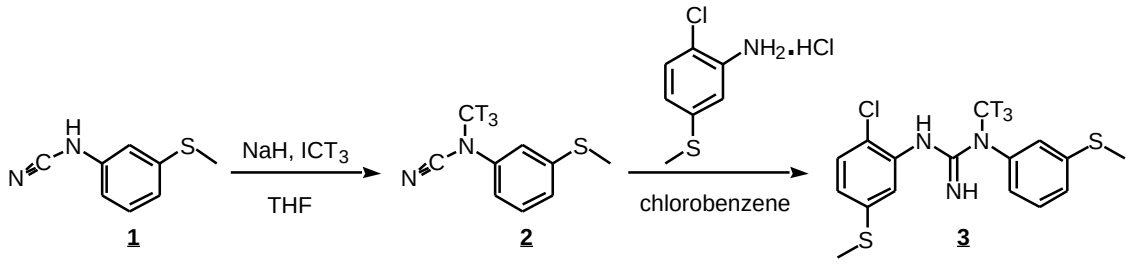


Figure 1. NMDA receptor non-competitive antagonists.

Results and discussion

The preparation of **3**, [$^{3}H_{3}$]CNS-5161, was accomplished in two steps by the pathway shown in Scheme 1, an adaptation of the synthesis described by Hu et al.[13] The major change to the original synthesis minimized the use of

halogenated solvents thereby limiting the amount of mixed radioactive waste produced.



Scheme 1. Preparation of [$^3H_3$]CNS-5161.

The intermediate N-[$^3H_3$]methyl-3-(thiomethylphenyl)cyanamide **2** was achieved by the addition of tritiated iodomethane{[14, 15] to 3-(thiomethylphenyl)cyanamide **1**, previously treated with sodium hydride. Excess non-radioactive iodomethane was added to the reaction to force the methylation to completeness, eliminating the need to chromatographically purify **2**. This process, however, reduced the specific activity from near theoretical 85 Ci mmol^{-1} to 66 Ci mmol^{-1}. The yield for this reaction was nearly 77% with the unreacted CT_3I removed during the evaporation process.

Reaction of **2** with 2-chloro-5-thiomethylaniline hydrochloride provided the guanidine compound [$^3H_3$]CNS-5161, **3**. A 5-6 fold stoichiometric excess of 2-chloro-5-thiomethylaniline hydrochloride was found to be necessary for guanidine formation. Purification through 2 HPLC columns gave **3**. with a

final specific activity of 66 Ci $mmol^{-1}$ and greater than 96% radiochemical purity. The yield for this reaction was ~12%. The final product was stored at a concentration of 1 mCi mL^{-1} in ethanol at -18 °C. Ten percent radiolytic decomposition was noted over a period of approximately nine months of storage.

Conclusion

The modified two step reaction sequence produced the desired tritiated guanidine product in an overall yield of 9% with a specific activity of 66 Ci $mmol^{-1}$. The final achievable specific activity could be improved by eliminating the use of non-radioactive iodomethane. This comes at the cost of increased handling associated with an extra chromatographic step. The final compound was suitable for *in vitro* and *in vivo* assessment of NMDA receptor function.

Experimental

General

All chemicals and solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification. Solvents were

dried by standard techniques. All reactions were performed under a nitrogen atmosphere. All NMR spectra were recorded using a Bruker DMX-300 MHz spectrometer at 320 MHz for ^{3}H. HPLC was carried out using a Waters 600E multisolvent delivery system with in-line UV monitoring of the effluent at 260 nm using a Hewlett Packard 1040A LC spectrometer and radioactivity detection using an in-line IN US beta RAM.

Tritium gas was procured from the Savannah River DOE complex and contained 98% $^{3}H_2$ and 2% $^{2}H^{3}H$. The purity of the reaction products was determined by radio-HPLC and ^{3}H NMR spectroscopy. The specific radioactivity of the tritiated products was determined by liquid scintillation counting (Packard Tricarb 1500) of the isolated HPLC peak effluents, as previously described.[16] Proton and tritium NMR analyses were also used to verify the specific radioactivity of the product.

[$^{3}H_3$]methyliodide. A solution of n-butyl lithium in hexane (1.6 M, 187 µL, 0.3 mmol) was rapidly stirred in the presence of an atmosphere of carrier-free tritium gas. Tetramethylethylenediamine (TMEDA) was added and after stirring for 20 minutes volatiles were removed. The residue was solvated with 500 µL THF followed by the addition of aluminum tribromide solution at

ambient temperature (250 µL in 1 mL THF) to give $LiAlT_4$. After 10 minutes carbon dioxide (1.5 mL) was injected and the reaction stirred for three hours. Hydrogen iodide (57%; 150 µL) was added and the reaction heated to 170 °C for 1 hour then cooled to ambient temperature.

Preparation of N-[$^3H_3$]methyl-3-(thiomethylphenyl)cyanamide **2**. Tritiated iodomethane was distilled across to a flask containing an ice-cooled suspension of cyanamide **1** (2.8 mg, 0.017 mmol) and sodium hydride (0.8 mg, 0.033 mmol) in THF, that had previously been heated to reflux for three hours. The reaction was allowed to warm to ambient temperature and stirred overnight. Non-radioactive iodomethane (5 µL, 0.08 mmol in 200 µL THF) was then added to facilitate complete methylation. Methanol (100 µL) was added at 0°C to quench the reaction and the solvent removed under reduced pressure. The oil was transferred to an extraction tube using water (2 mL) and diethyl ether (10 mL). The ether was separated, dried ($MgSO_4$) and the solvent removed under a stream of nitrogen to give cyanamide **2** in 77% yield. **^{3}H-NMR** (320 MHz; THF-d8) ∂ [ppm] 3.21 (NC^3H_3, s, 97% $^3H_3$, 2% $^3H_2{}^1H$ and 1% $^3H^1H_2$)**.**

N-(2-chloro-5-methylthiophenyl)-N'-(3-methyl-thiophenyl)-N'-[$^{3}H_3$]methylguanidine ***3***. A reaction vessel was charged with 825 mCi of the tertiary cyanamide **2**. To this was added an excess of aniline hydrochloride (20.8 mg, 0.098 mmol). A slurry was produced by the addition of 200 µL anhydrous chlorobenzene. The reaction was heated to 165°C for 24 hours. The chlorobenzene was removed and the residue was dissolved in 1 mL of methanol. Purification of **3** was performed using two HPLC columns. Initial separation of **3** from **2** was carried out on a Zorbax CN (4.6 x 250 mm; 65/35 methanol/ 0.1N ammonium formate; flow rate 1.2 mL min^{-1}; t_r **3** =11 min, **2** = 6.25 min) The 11 min peak was collected, concentrated then further purified using an HP Agilent LC-18 column (4.6 x 250 mm; 65/35 methanol/ 0.1N ammonium formate; flow rate 1 mL min^{-1}; t_r **3** =9 min). Lyophilization of the solvent gave 100 mCi of [$^{3}H_3$]CNS-5161, **3** (12% yield). The radiochemical purity of **3** was determined to be >96% by analytical HPLC (HP Agilent LC-18 column, 4.6 x 250 mm; 65/35 methanol/ 0.1N ammonium formate; flow rate 1 mL min^{-1}). **^{3}H-NMR** (320 MHz; CD_3CN) ∂ [ppm] 3.28 ($NC^{3}H_3$, s).

Acknowledgements

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